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Administration of a Highly Attenuated, Live Respiratory Syncytial Virus Vaccine to Adults and Children

PETER F. WRIGHT,[1]* ROBERT B. BELSHE,[2] HYUN W. KIM,[3] LEE P. VAN VORIS,[2] AND ROBERT M. CHANOCK[4]

Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232[1]; Department of Medicine, Marshall University School of Medicine, Huntington, West Virginia 25701[2]; Children's Hospital, National Medical Center, Washington, D.C. 20010[3]; and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md. 20205[4]

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A highly attenuated respiratory syncytial virus (RSV) experimental vaccine, RSV *ts*-2, was sequentially evaluated in adults, seropositive children, and finally, fully susceptible seronegative children. The vaccine was administered intranasally in doses ranging from $10^{5.2}$ to $10^{6.3}$ PFU/ml. In both adults and children, the vaccine proved to be poorly infectious. Although poor infectivity would not have been predicted from tissue culture studies of RSV *ts*-2 growth, the human experience closely parallels the experience in a series of animal models, including the chimpanzee. The poor infectivity of this RSV vaccine virus preparation suggests that the postulated defect in the RSV *ts*-2 fusion protein may be important in determining in vivo infectivity of RSV.

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia in infants and young children (4). The major effort at prevention of serious lower respiratory disease caused by RSV has been directed toward the development of live, attenuated vaccines that would stimulate both local respiratory and systemic immunity. The first such vaccine candidate, RSV mutant *ts*-1, was clearly attenuated, but the vaccine was not genetically stable when administered to seronegative children (10). Subsequent work has focused on increasing the stability of the temperature-sensitive mutations which contribute to attenuation and simultaneously serve as vaccine virus markers. The use of chimpanzees and other primates as sensitive experimental hosts for determining the degree of attenuation of RSV vaccines has allowed increasingly sophisticated screening of vaccine candidates before administration to humans. A highly attenuated virus, RSV mutant *ts*-2, emerged from a series of laboratory and animal model evaluations as a promising candidate vaccine. In the present studies, RSV *ts*-2 was administered sequentially to adults, seropositive children, and finally, to seronegative children (Table 1). As in the primate host, RSV *ts*-2 proved to be a poorly infectious and weakly immunogenic virus in humans.

Two virus suspensions containing RSV *ts*-2 were prepared by Flow Laboratories, Inc. according to standard vaccine production protocols. They were shown to be free of adventitious agents and had titers of $10^{4.5}$ and $10^{6.3}$ PFU/ml. Virus in these suspensions produced nonsyncytial plaques typical of RSV *ts*-2 (3).

Trials were carried out in four settings. Adults were studied at the University of Maryland, Baltimore, using a study design described for other adult vaccine trials (7). Children were studied at Children's Hospital National Medical Center, Washington, D.C., Marshall University, Huntington, W.V., and Vanderbilt University, Nashville, Tennessee, in settings previously described (5, 10). Adults and children were observed for 12 to 15 days after vaccine administration with daily cultures for virus in HEp-2 or HeLa cells (10). Antibody response was evaluated with complement fixation, plaque neutralization, or enzyme-linked immunosorbent assay at the cooperating centers (5, 9, 10).

Adults. A total of 14 young adults selected for low-neutralizing nasal-wash antibody to RSV were inoculated intranasally with $10^{5.2}$ PFU of RSV *ts*-2 per ml in a volume of 5 ml. RSV virus was not recovered during nasal washings collected on 12 consecutive days after vaccine administration. Two individuals developed signs and symptoms of upper respiratory illness during the study. A rhinovirus was recovered from one of the ill volunteers. None of the volunteers developed a rise in serum plaque neutralization antibody by days 12 or 26 postchallenge. However, two volunteers developed a fourfold rise in nasal-wash neutralizing antibody titer (corrected to 20 mg of immunoglobulin A per dl), one of whom had a fourfold rise in serum complement fixation antibody titer. Thus, 2 of 14 volunteers

TABLE 1. Summary of studies with RSV *ts*-2 mutant in experimental animals and humans

Species	Inoculum ($TCID_{50}$[a])	No. studied	No. with virus recovery	No. with serological response	No. with illness	Reference
Rodent						
Hamster	$10^{6.5}$	16	5/12	All tested	None	11
Cotton rat	$10^{4.6}$	14	0/26	None	None	Unpublished data
	$10^{5.7}$	12				
Primate						
Owl monkey	$10^{4.3}$	2	1/4	2/4	None	8
	$10^{5.3}$	2				8
Chimpanzee	10^{6}	2	6/9	5/9	None	
	$10^{5.6}$	4				
	$10^{6.7}$	2				
	$10^{7.6}$	1				
Humans						
Adults	$10^{5.2}$	14	0/14	2/14	None	Present paper
Seropositive children	$10^{6.3}$	20	0/20	0/20	None	
Seronegative children	$10^{6.3}$	7	1/7	2/7	None	

[a] $TCID_{50}$, 50% tissue culture infective dose.

appeared to have been infected with the RSV *ts*-2 mutant.

Children. Trials were subsequently carried out with 20 seropositive children, aged 11 months to 5 years. A higher titer vaccine suspension of $10^{6.3}$ PFU/ml was prepared for the pediatric studies. It was administered in a volume of 0.5 ml intranasally. Of 20 volunteers, 1 shed RSV *ts*-2 on day 1 after vaccination. Otherwise, virus was not recovered from daily nasal-wash specimens collected for 15 days after vaccine administration. A total of 15 children had a prechallenge serum neutralizing (plaque reduction) antibody titer of greater than 1:100, whereas 5 had a relatively low serum antibody titer in the range of 1:20 to 1:100. None of these seropositive children developed a rise in serum neutralizing antibody. Nasal-wash antibody responses were not determined.

Nine seronegative children, aged 6 to 27 months, were studied at Vanderbilt University and Marshal University. Vaccine was administered in a volume of 0.5 ml (five children) or 1.0 ml (four children). One vaccinee (vaccinee 1, Table 2) shed RSV on days 12 and 14 postvaccination. The recovered virus produced syncytial plaques. As wild-type RSV was not detectable in the community, these isolates probably represented revertant *ts*-2 virus, as previously described in the primate models (1).

No clinical illness ascribable to RSV *ts*-2 was seen in the pediatric trials with either seropositive or seronegative children, although minor intercurrent illness was observed in several trials attributable to the isolation of adenovirus (nine children) and parainfluenza types III (one child) and II (one child). In one trial, wild-type RSV was introduced before vaccination by a child who had a mild upper respiratory illness upon admission to the trial. A single day's contact with this child was sufficient to infect three seronegative vaccinees and one seropositive child. The three seronegative children became ill, whereas the seropositive child underwent an asymptomatic infection (Table 2).

Two of the seven seronegative vaccinees not exposed to wild-type RSV developed a seroresponse demonstrable by plaque neutralization or enzyme-linked immunosorbent assay or both. The responses were of relatively low magnitude and short duration (Table 2). A comparison could be made with the higher antibody responses in vaccinees undergoing natural infection (Table 2).

Each of the seven seronegative vaccinees was followed through for at least one RSV season subsequent to vaccination (Table 2). RSV was recovered from four children who developed definite respiratory tract illness. One additional child developed an RSV seroresponse without an associated illness. It is of interest that two of the five children who underwent natural infection with RSV had previously developed a seroresponse to the RSV *ts*-2 mutant.

RSV *ts*-2 replicates to a high titer in tissue culture at the permissive temperature and is somewhat leaky in that virus replication occurs to a limited extent even at 39°C (3). Thus, from in vitro studies, it would not appear to be as highly defective as RSV *ts*-1, a previously evaluated mutant (10). However, RSV *ts*-2 was regarded as a highly attenuated vaccine candidate based on studies in rodents and primates (Table 1).

The data from our evaluation of the *ts*-2 mutant in humans closely parallels our experi-

TABLE 2. Summary of evaluation of RSV *ts*-2 in 11 seronegative infants and children

Vaccinee	Age (mo) at vaccination	Antibody titer at following time by indicated test[a]				Subsequent natural infection					
		Prevaccination		Postvaccination		Naturally infected with RSV	Illness with natural infection	Pre-epidemic antibody titer		Post-epidemic antibody titer	
		ELISA	Neut	ELISA	Neut			ELISA	Neut	ELISA	Neut
1	11	<50	<4	709[b]	13[b]	Yes[c]	URI,[d] mild wheezing	60	—[e]	8588[b]	—
2	9	<50	<4	<50	6	Yes[c]	URI, mild wheezing	<50	—	6318[b]	—
3	8	<50	<4	<50	<4	Yes[c]	URI, bronchiolitis	<50	—	75[b]	—
4	6	68[f]	12[f]	50	5	Yes[c]	URI	<50	—	203[b]	—
5	24	<50	<16	<50	<16	No	—	—	<4	—	<4
6	14	<50	4	<50	15[b]	Yes	Undetermined	—	4	—	100[b]
7	12	<50	4	<50	5	No	—	—	4	—	<4
8	14	<50	<4	3,092[b]	350[b]	Yes[c]	Bronchiolitis				
9	21	<50	<4	3,046[b]	110[b]	Yes[c]	Febrile URI				
Controls											
10	30	<50	<16	<50	<16	No	—	—	5	—	4
11	9	<50	5	294[b]	500[b]	Yes[c]	URI				

[a] ELISA, Enzyme-linked immunosorbent assay; Neut, neutralizing antibody. The boxes indicate natural RSV introduced into group at initiation of trial (3 days before vaccine administration).
[b] Significant antibody rise.
[c] Wild-type RSV recovered at the time of natural infection.
[d] URI, Upper respiratory illness.
[e] —, Not done.
[f] Probable maternal antibody.

ence in experimental animals and reinforces the utility of animal models, particularly chimpanzees, in the screening of potential RSV vaccine candidate strains. Unfortunately, the RSV *ts*-2 mutant must be judged to be overly attenuated and thus is not suitable for use in immunoprophylaxis of RSV illness, since susceptible young children could not be uniformly infected with a high dose of virus.

The site of the *ts*-2 defect was elucidated in previous studies in which it was shown that this mutant was defective in adsorption or penetration or both of host cells at the restrictive temperature (2). This is consistent with a defect in the putative fusion protein in RSV. Lending further support to a defect in the fusion protein of RSV is the nonsyncytial nature of plaques produced by the *ts*-2 mutant. The importance of paramyxovirus fusion protein in initiating viral infection has been emphasized recently by Merz et al. (6). The overattenuation of the *ts*-2 mutant lends support to the importance of the cell entry function mediated by RSV fusion protein.

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